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Study of physico-chemical property of bioenzymes produced from organic household waste and their application in daily life

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ABSTRACT

Bioenzymes (also known as eco-enzymes or garbage enzymes) was prepared using organic waste like peels of citrus, pineapple, banana, neem leaves, marigold and rose petals along with jaggery, water and yeast. The mixture was allowed to ferment for about one month in an air-tight plastic bottle. The physical characteristics like pH, total dissolved solids (TDS), colour and smell were studied for the produced bioenzymes. The phytochemical test of bioenzyme revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, quinones, saponins and cardenolides in them which are known for their antibacterial, antifungal, antiviral, anti-inflammatory, anti-feedant, insecticidal and foaming properties. Citrus and pineapple bioenzymes were found effective for use as cleaning agent, while banana and neem bioenzymes were found effective for use as natural fertilizers. Neem bioenzymes also showed positive results when used as a natural pesticides. To study the antifungal properties of bioenzymes simple bread mould experiment was conducted in which neem bioenzymes were found to be most potent in retarding the fungal growth.

Key words- bioenzymes, secondary metabolites, anti-inflammatory, insecticidal, anti-fungal.

1. INTRODUCTION

In India, with an increase in the growth of the population, the generation of waste has also been increased, which not only affects the economy of the country but also has an adverse effect on the environment (Yadav & Singh, 2021). It is reported that India produces 147,613 metric tonnes of solid waste per day (Singh, 2020). Organic wastes are a major component of municipal solid waste, which mainly comprises of wet kitchen waste like fruits and vegetable peels, food waste, landscape and pruning wastes (Rasit and Kuan, 2018; Akusu et al., 2019). They become a mess when dumped directly into landfills because these wastes undergo anaerobic decomposition by the action of microorganisms and produces

landfill gas. These gases contributes to global warming and are responsible for change in climatic conditions.

In 2006, Dr. Rosukon Poompanvong from Thailand developed a complex solution fabricated from organic solid waste and termed it "Garbage Enzyme" (Novianti & Muliarta, 2021). Garbage Enzyme is also known by most people as Bio-Enzyme or Eco-Enzyme. It is a gloomy brown solution with vinegar like aroma. It mainly consists of alcohol, acetic acid, vitamins, minerals, salts, enzymes like amylase, cellulase, lipase and protease, amino acids, hormones and some good microbes. Bioenzyme is produced by the simple fermentation of organic wastes like fruits and vegetable peels, flowers or leaves accompanied by molasses and water. All the above ingredients are mixed in the ratio of 3:1:10 ratio that is 3 part of organic waste, 1 part of molasses and 10 part of water. This solution is then allowed to ferment for 3 months (Samriti et al., 2019).

Fermentation is defined as any metabolic process which involves the use of microbes like yeast and bacteria to break down sugar and starch into alcohol and acid via the action of enzymes. Here, firstly the naturally occurring yeast and bacteria present on the surfaces of fruit and vegetables in the absence of oxygen breaks down the sugar molecules such as glucose, sucrose and fructose into a cellular energy adenosine tri-phosphate (ATP). Ethanol and carbon dioxide are produced as a by-product during this process. However, some species of yeast like *Saccharomyces cerevisiae* and *Zymomonas mobilis* (a gram negative, facultative anaerobic bacteria) can produce ethanol even in the presence of oxygen. Then the alcohol is further oxidized to acetic acid by the help of acetic acid bacteria which are often found abundantly in air, water, fruits and vegetables residue, fermented product containing alcohol and in food materials containing sugar (Al-Maqtari et al., 2019).

The chemical properties of these bioenzymes are similar to those found in other biological systems. It can be used as a natural cleaning agent in the home, as an agricultural fertilizer, insecticide, herbicide, and pesticide, for the purification of grey water and air, for drain de-clogging, road construction, and improving soil quality (Sethi et al., 2021). It can also be used as a bio-remediation based agent for the removal of heavy metals (Ingle & Saler, 2021) from contaminated soil (Wei et al., 2020) and for bio-catalytic remediation of oil contaminated soil (Bulai et al., 2021; Promise et al., 2020).

In this study our aim was to produce bioenzymes using organic household waste and to study their physicochemical properties and applications in daily life.

2. MATERIALS AND METHODS

2.1. Preparation of Bioenzymes:

For the preparation of bioenzymes organic waste like fruits peels (citrus, pineapple, banana), flowers (rose & marigold), neem leaves, molasses (jaggery), water, plastic bottles and dry active yeast (of solar sales company) were taken. Fruit peels/flowers/leaves were chopped into smaller pieces with the help of a scissors. 1 part of jaggery, 3 parts of organic waste & 10 parts of water were taken. Then all the ingredients were put into a plastic bottle & to this one gram of yeast was added. After this, bottle mouth was capped tightly & gently shaken till all the jaggery was completely dissolved into the water. Bottle was then kept in cool, dry & dark place at the room temperature & allowed to ferment for one month. During the first week, gas formed was released on daily basis for about at least two times a day by slowly opening the bottle cap. During the second week, gas production was low & gases were released on the alternate days or if necessary. Then the mixture was left to ferment for another two weeks. After one month, garbage enzyme solution is filtered using a sieve. The residue (left-over) can be used again for the production of next batch of bioenzyme or it can be used as a scrubber for cleaning purposes or it can be used as a fertilizer (Nazim and Meera, 2015).

2.2. Physico-chemical properties of Bioenzymes:

Physical characteristics of bioenzymes like colour, smell, pH, total dissolved solids (TDS) were studied by sight, smell, pH strip and TDS meter (HM Digital) respectively.

Biochemical test were done to confirm the presence of alkaloids, flavonoids, quinones, saponins, tannins, and cardenolides.

2.2.1. Alkaloids estimation: The test was performed following the protocol mention in Vama and Cherekar, 2020. To the 2 ml of bioenzyme samples, 1ml of freshly prepared Wagner's reagent (0.25g of iodine and 0.4g of potassium iodide in 20ml of distilled water) was added and observed for the formation of reddish/brown precipitate.

2.2.2. Flavonoids estimation: The test was performed following the protocol mention in Kumar et al., 2014. To the 2ml of bioenzymes samples, few drops of freshly prepared 10% lead acetate (2g of lead acetate in 20ml of distilled water) was added and observed for the formation of dark yellow or orange colour.

2.2.3. Quinones estimation: The test was performed following the protocol mention in Ugochukwu et al., 2013. To the 2ml of bioenzyme samples, 1-2 ml of concentrated HCl was added drop wise and observed for the formation of yellow colour.

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2.2.4. Saponins estimation: The test was performed following the protocol mention in Vama and Cherekar, 2020. To the 2 ml of bioenzymes sample, 2-3 ml of distilled water was added and shaken vigorously and observed for the formation of foam.

2.2.5. Tannins estimation: The test was performed following the protocol mention in Gonfa et al., 2020. To the 1 ml of bioenzyme samples, 1ml of freshly prepared 5% ferric chloride solution was added and observed for the formation of blackish green colour.

2.2.6. Cardenolides estimation: The test was performed following the protocol mention in Vama and Cherekar, 2020. To the 5ml of crude bioenzyme samples, 2ml of glacial acetic acid along with 1 drop of 5% ferric chloride solution was added in a test tube. This was precisely layered with concentrated sulphuric acid for the formation of brown ring at interface.

2.3. Application study of bioenzymes:

2.3.1. Study of cleaning activity of bioenzymes produced from citrus and pineapple

(i) Citrus bioenzyme was applied on to the tap and tiles for the removal of lime scales, hard water stains and dirt from it. The solution was applied for about half to one hour. ii) To one bucket half filled with water, 5 ml of citrus and pineapple bioenzyme (concentrated) was added for the removal of soil dirt, oil, fat and grease stains from the white cloth and was kept overnight. (iii) Citrus and pineapple bioenzyme residue was grinded in a mixer grinder to obtain a smooth paste. This paste was applied on to the copper & silver utensils for cleaning and was kept overnight.

2.3.2. Study of fertilizing activity of bioenzymes produced from neem and banana

The use of bioenzyme as a fertilizer was studied on Cosmos plant *(Cosmos bipinnatus).* Three Cosmos plant of same height were taken from a nursery near AD Kanya Inter College, Bank road, Gorakhpur. Then it were planted in three different pots labeled as control, neem bioenzyme and banana bioenzyme respectively. Pot labeled as control was irrigated with water, pot labeled as neem bioenzyme was irrigated with neem bioenzyme in 1:1 dilution and the pot labeled as banana bioenzyme was irrigated with banana bioenzyme in 1:1 dilution respectively. Irrigation with water, neem and banana bioenzyme were done in equal quantities for 1 month at every alternate day or with a gap of 2 days.

2.3.3. Study of pesticidal activity of bioenzyme produced from neem

The use of bioenzyme as a pesticide was studied on Tulsi plant *(Ocimum tenuiflorum)* using neem bioenzyme. The plant was already infected by aphids & neem bioenzyme in 1:1 dilution was sprayed on the affected areas at every alternate days for 1 months.

2.3.4. Test for antifungal activity of bioenzymes

To study the anti-fungal property of bioenzyme samples a simple bread mold experiment was conducted. For this 7 slices of fresh bread and 7 plastic zipper bag were taken. 7 plastic bag were labeled as control, water, citrus, pineapple, banana, neem, marigold and rose. 1 slice of bread was sealed in control labeled plastic bag. The plastic bag labeled as water had bread slice sprayed with water. Plastic bag labeled as citrus, pineapple, banana, neem, marigold & rose contain bread sprayed with citrus bioenzyme, pineapple bioenzyme, banana bioenzyme, neem bioenzyme, marigold bioenzyme and rose bioenzyme respectively. (All the bioenzyme were taken in 1:1 dilution ratio). After this, all the plastic bags were sealed and were kept in a warm, dark place for about 10-12 days (https://www.education.com/science-fair/article/hand-soaps-sanitizers-prevent-bread-mold/).

3. RESULTS

3.1. Production of bioenzymes

After one month, peels got settled down which indicates that bioenzyme was ready for use and the solution was filtered using a sieve.

3.2. Physico-chemical properties of bioenzymes studied are listed in Table 1.

3.3. Application study of bioenzymes

3.3.1. Citrus and pineapple bioenzymes showed positive results when used as a cleaning agent (Fig.1).

3.3.2. Healthy growth of Cosmos plant was observed when neem and banana bioenzyme as a fertilizer was used (Fig.2).

3.3.3. Neem bioenzyme showed positive result as a pesticide when sprayed on Tulsi plant (Fig.3).

3.3.4. When diluted bioenzymes samples were sprayed on fresh slice of bread and incubated for about 10 days, it was observed that they were capable of inhibiting the fungal growth. (Fig 4).

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Table 1: Results of physico-chemical properties of bioenzymes

Physical Properties						
Bioenzymes	**pH**	**Total dissolved solids (ppm)**	**Colour**	**Smell**		
Citrus	1-2	159	Yellow	Orange fruit juice like		
Pineapple	1-2	218	Dark Orange	Pineapple fruit juice like		
Neem	5-6	180	Yellow-greenish	Unpleasant		
Banana	5-6	189	Dark Yellow	Unpleasant		
Rose	5-6	779	Reddish Brown	Vinegar like		
Marigold	5-6	116	Light Orange	Vinegar like		
Biochemical tests						
Bioenzymes	**Alkaloids**	**Flavonoids**	**Quinones**	**Saponins**	**Tannins**	**Cardenolides**
Citrus	+	+	+++	-	++++	-
Pineapple	++	++	++	-	+	-
Banana	+++	+	++++	-	+++	+
Neem	++	+	+	+	+++	+
Marigold	+	++++	+	-	++	-
Rose	+	+++	-	++	++++	-

++++ (Very strong positive), +++ (Strong Positive), ++ (Slightly less positive), + (Very less positive), - (Absent)

Figure 1: Before and after use of citrus and pineapple bioenzymes on taps, tiles, white cloth, copper and silver utensils.

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Figure 2: Effect of neem and banana bioenzyme on growth of Cosmos plant (*Cosmos bipinnatus*).

Figure 3: Neem bioenzyme helped to get rid of pests when sprayed on Tulsi plant (*Ocimum tenuiflorum).*

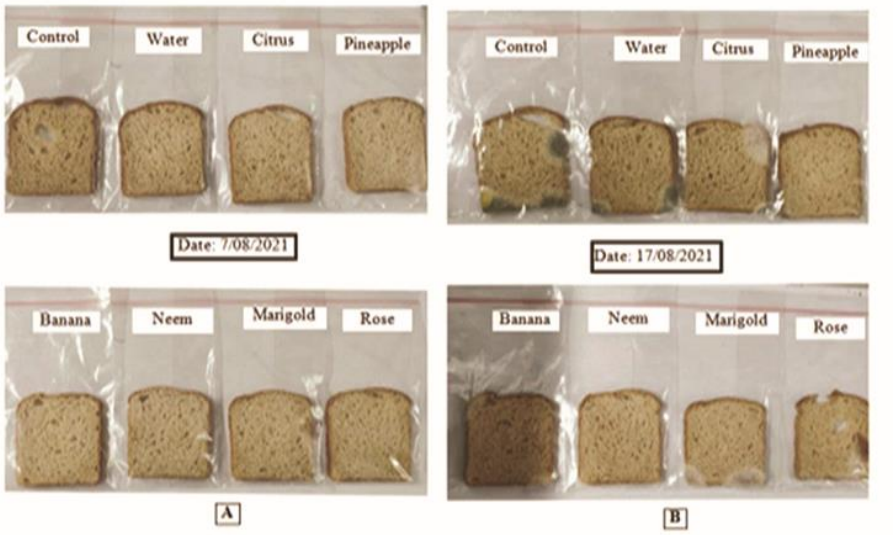

Figure 4: Result for anti-fungal activity. A) Before Incubation. B) After Incubation.

4. DISCUSSION

The preparation of bioenzyme was done by following the protocol listed by Srimathi N.et.al., 2020. Yeast was added during the preparation of bioenzyme because it speeds up the fermentation rate and reduces the time of the fermentation process. Jaggery is used as a source of sugar as it packed with minerals, vitamins and antioxidants. All peels, leaves, and flowers settled down after one month of fermentation, indicating that the bioenzyme is now ready to use (Srimathi N. et al., 2020). The fermentation of rose and marigold flowers took longer than a month because the petals did not settle down, but they were still harvested.

Bioenzymes contains secondary metabolites like alkaloids, flavonoids, quinones, saponins, tannins and cardenolides which are well known for their insecticidal, anti-feedant, anti-microbial, anti-oxidant and foaming properties (Mujeeb et al., 2014).

Citrus and pineapple bioenzymes were found to be helpful in eliminating lime scale, hard water stains, and debris from taps and tiles, as well as in cleaning of copper and silver utensils, due to the presence of organic acids such citric acid, malic acid, oxalic

acid, succinic acid, and glucaric acid (Srimathi N. et al., 2020). Citric acid, the active ingredient in cleaning solutions, works as a chelating agent and aids in the removal of lime scale from taps, tiles, and boilers, as well as hard water stain from glasses and the dissolution of rust from steel(Behera et al., 2021).

Long-term usage of inorganic fertilizer kills the beneficial bacteria in the soil, making plants more susceptible to disease. Bioenzyme is utilized as an organic fertilizer to solve these difficulties. Banana peels and neem leaves were used as a natural fertilizer for the growth of Cosmos plant. Both of them act as a growth stimulant for plant. Macronutrients present in them are potassium (in high amount), magnesium, calcium, sodium and phosphorous whereas the micronutrients present are iron, zinc, manganese and copper. Potassium helps in growth and development of plant by enhancing the nutritional uptake by plants. Phosphorous is an essential element of cell membrane, nucleic acid, proteins and is needed for certain major reaction. Nitrogen is available to soil in form of ions and is required for the growth of plant. Beside these minerals, bioenzymes from these peels also contain vitamins, essential amino acid and plant growth hormones which provide proper nutriment to the soil and helps in the growth of plants. They also help plants to fight against abiotic condition like drought and stress (Sethi et al., 2021).

Neem bioenzymes were found to be effective against pest on Tulsi plant. The main active compound found in neem leaves are azadirachtin, nimbolide and salannin. Azadirachtin is a composite tetranortriterpeniod limonoid which is having pesticidal, anti-feedant, oviposition inhibitor and repellent properties. It interrupts with the growth and development of insect and hinders with the synthesis of insect molting hormone “ecdysome” and stops the molting and metamorphosis process causing the death of the insect. Two active element of nimbolide are nimbolide B and nimbic acid which are known to have herbicidal properties. Salannin act as insect growth regulators and mimics like insect hormones and damages the growth and reproduction of insects. It also act as an anti-feedant. (Chaudhary et al., 2017).

Molds need water, nutrients and warm condition to grow. Bread samples sprayed with different bioenzyme samples showed less fungal growth due to presence of propionic acid, acetic acid and alcohol. They prevent the growth of bacteria that cause spoiling of food and slow down mold developments. Alcohol inhibits the development of mold by increasing the permeability of membrane due to which membrane gets leaky and all the solutes get leaked out, ultimately leading cell lysis (Legan, 1993).

5. CONCLUSION

Bioenzymes are produced by fermenting organic wastes. Due to its wide range of applications in households, agriculture, and the environment, they are also known as a multi-purpose liquid. They are known for their antibacterial, antifungal, anti-feedant, insecticidal, and foaming properties due to the presence of secondary metabolites in them, and can be used as a cost-effective, degradable, organic, and environmentally friendly alternative to all commercially available chemical products. It does not pollute the water or soil system, emits no toxic fumes, and has no harmful effects on humans or animals.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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